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POTENT AND SELECTIVE INHIBITORS OF THE PROTEASOME: DIPEPTIDYL BORONIC ACIDS

Julian Adams,* Mark Behnke, Shaowu Chen, Amy A. Cruickshank, Lawrence R. Dick, Louis Grenier, Janice M. Klunder, Yu-Ting Ma, Louis Plamondon, and Ross L. Stein

ProScript, Inc., 38 Sidney Street, Cambridge, MA 02139, U.S.A.

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Abstract. Potent and selective dipeptidyl boronic acid proteasome inhibitors are described. As compared to peptidyl aldehyde compounds, boronic acids in this series display dramatically enhanced potency. Compounds such as **15** are promising new therapeutics for treatment of cancer and inflammatory diseases.
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The 26S proteasome is the multi-catalytic protease responsible for the majority of intracellular protein turnover in eukaryotic cells, including proteolytic degradation of damaged, oxidized or misfolded proteins, as well as processing or degradation of key regulatory proteins required for various cellular functions.[1] Constituting the catalytic core of this complex is the 20S proteasome, a multi-subunit complex of approximately 700 kDa molecular weight. While serving an essential physiological role, the proteasome is also responsible for the inappropriate or accelerated protein degradation that occurs as a result or cause of pathological conditions in which normal cellular processes become disregulated. One notable example is cancer, in which the unregulated proteasome-mediated degradation of cell cycle regulatory proteins, including cyclins, cyclin dependent kinase inhibitors, and tumor suppressor genes, results in accelerated and uncontrolled mitosis, thereby promoting cancer growth and spread.

We have sought to inhibit the proteasome enzymatic function in order to arrest or blunt disease progression in disease states such as cancer or inflammation.[2] The earliest reported proteasome inhibitors included peptidyl aldehydes such as **1**, which were shown to inhibit the chymotryptic-like activity of the complex.[3] As recently revealed by X-ray crystallography,[4] the aldehyde inhibitors form hemi-acetal adducts with the active site threonine nucleophile of β-subunits. Preliminary optimization of the tripeptide aldehyde series of inhibitors based on **1** (Table 1) revealed that leucine is preferred at the P1 position. Introduction of large hydrophobic residues, such as naphthylalanine, at P2 or P3 greatly enhances potency (compounds **6** and **7**).

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Peptidyl aldehydes, albeit potent inhibitors of proteasome-mediated proteolysis, are also potent inhibitors of thiol proteases such as cathepsin B and calpains.[5] Furthermore, the substituent adjacent to the aldehyde is not configurationally stable, due to the acidity of the α-proton. Finally, tripeptidyl aldehydes are expected to display poor metabolic stability and bioavailability, limiting their in vivo utility.

Table 1. Proteasome Inhibitory Activity of Peptidyl Aldehydes

Cbz—P3—P2—P1—H

Compound	P3	P2	P1	K_i (nM)[6]
1	Leu	Leu	Leu	4
2	Leu	Leu	Ile	130
3	Leu	Leu	Ala	210
4	Leu	Leu	Gly	3,800
5	Leu	Leu	2-Nal	25
6	2-Nal	Leu	Leu	0.24
7	2-Nal	1-Nal	Leu	0.015

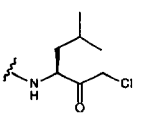
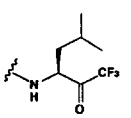
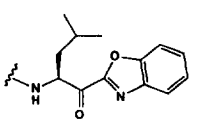
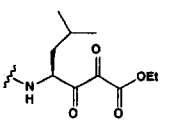
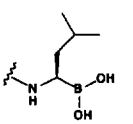
A number of aldehyde replacements were examined in the attempt to overcome these shortcomings (Table 2).[7] Although often exploited as serine protease inhibitors, chloromethyl ketones and trifluoromethyl ketones did not inhibit proteasome activity (e.g., **8** and **9**). Modest inhibition was observed with ketobenzoxazole **10** and diketo ester **11**. However, the breakthrough came with synthesis of boronic acid **12**, which exhibits a 100-fold enhancement in potency relative to **1**. An independent report of peptide boronic acid proteasome inhibitors appeared recently,[8] but interestingly, in that case no enhancement in potency of the boronic acids as compared to the corresponding aldehydes was observed.

The activity of peptidyl boronic acids as serine protease inhibitors is well-documented,[9] and is attributable to the availability of an empty *p*-orbital on boron, which is well-suited to accept the oxygen lone pair of the active site serine residue. By analogy, it is presumed that the boronic acid compounds also form stable tetrahedral intermediates with the *N*-terminal threonine residues of the catalytically active proteasome

β–subunits.[10] By contrast, such an interaction is not possible with cysteine proteases, owing to the very weak bond between sulfur and boron.[11] In fact, compound **12** inhibits cathepsin B with a K_i of only 6.1 μM, representing a 200,000-fold selectivity for the proteasome.

Table 2. Aldehyde Surrogates

Cbz—Leu—Leu—X

Compd	X	K_i (nM)[6]
8		22,000
9		1,400
10		214
11		45
12		0.03

With potencies of tripeptidyl boronic acids in the low picomolar range, it was possible to truncate the molecule and still retain good inhibitory activity (Table 3). Whereas very large hydrophobic P2 residues, such as naphthylalanine, are required to achieve reasonable potency in the dipeptidyl aldehyde series,[12] dipeptidyl boronic acids with phenylalanine at this position exhibit sub-nanomolar potency.

Dipeptidyl boronic acids such as **15** offer the practical advantages of reduced molecular weight and simplified synthesis (see below). In addition, these compounds exhibit extremely high selectivity for the proteasome over common serine proteases (Table 4). Enzymes such as chymotrypsin and elastase require S3 and S4 subsite binding for optimal activity,[13] a condition not fulfilled with the dipeptide inhibitors, while thrombin has a preference for basic residues at P1[14] and is not inhibited by compounds with leucine boronic acid at that position.

Table 3. Comparison of Aldehyde and Boronic Acid Proteasome Inhibitors

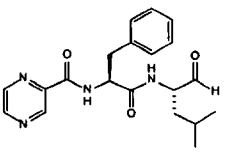
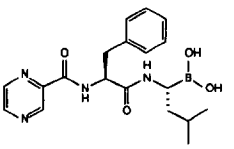
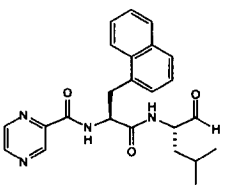
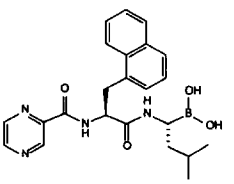
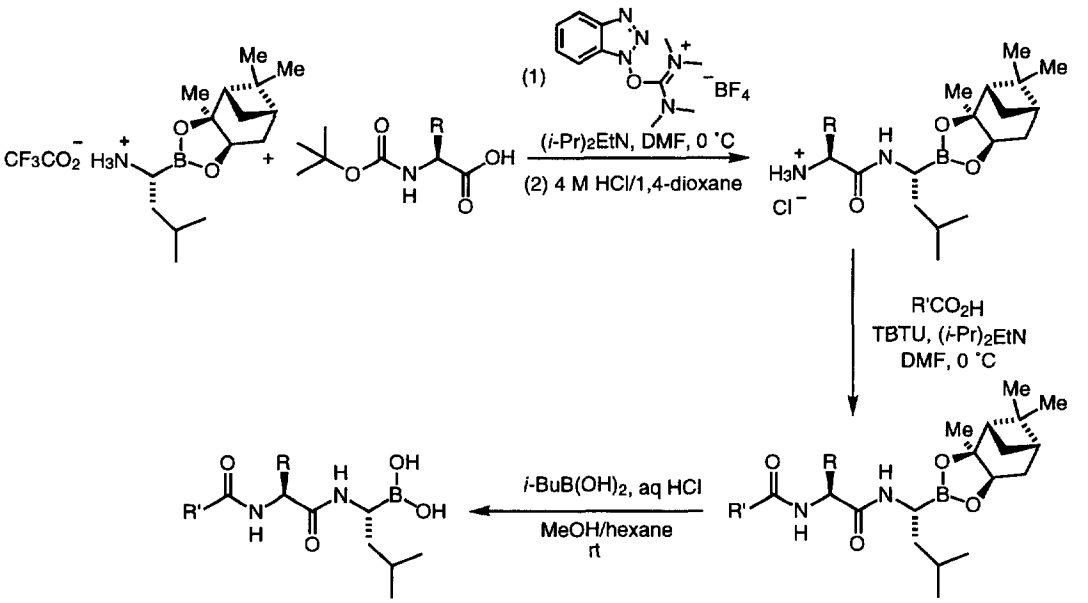
Compd	X	K_i (nM)[6]	Compd	X	K_i (nM)[6]
13		1,600	**15**		0.62
14		97	**16**		0.18

Table 4. Enzyme Inhibitory Profile of Compound **15**

Enzyme	K_i (nM)
20S Proteasome	0.62
Human leukocyte elastase	2,300
Human cathepsin G	630
Human chymotrypsin	320
Thrombin	13,000

Synthesis of the dipeptide boronates reported here was accomplished by adaptation of previously reported methods,[15] as illustrated in Scheme 1. The pinanediol ester of leucine boronic acid[16] was coupled with an *N*-Boc protected amino acid in the presence of (TBTU). Deprotection and *N*-acylation then afforded the dipeptide boronate ester. Boronic acid deprotection was accomplished by two-phase transesterification with isobutyl boronic acid, with the desired product being isolated by extractive workup. The application of this methodology to large scale synthesis of **15** will be reported separately.

Scheme 1



In addition to displaying potent inhibition of proteasome enzymatic function in vitro, the peptidyl boronic acids typified by **15** also profoundly impact proteasome-dependent physiological processes in culture and in vivo.[17] Antitumor and antiinflammatory efficacy has been achieved with these agents in a number of different animal models.[17] Compounds such as **15** thus represent promising new therapeutics for treatment of cancer and inflammatory diseases.[18]

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18. Preliminary information about compound **15** and its proteasome inhibitory activity has been disclosed previously. See reference 2.